J. Mol. Biol. (1965) **12**, 266–279

Coliphage MS2 containing 5-Fluorouracil

I. Preparation and Physical Properties

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(*Received 18 January 1965*)

5-Fluorouracil inhibits the formation of MS2 measured as infective units or physical phage particles. Addition of the base analog during the first 13 minutes after infection has a greater effect than addition at a later time. Whereas 30 μg/ml. 5-fluorouracil added at five minutes after infection results in a marked decrease in progeny phage particles, later addition of FU† allows up to 50% normal yield of physical phage, some of which contains FU. FU phage has a greater buoyant density in CsCl than normal MS2, the density being directly related to the degree of replacement of uracil by FU. This density shift is due to an increased buoyant density of FU-RNA, measured in cesium sulphate. FU phage and FU-RNA have normal sedimentation coefficients, and the T_m of FU-RNA, in which 80% of the uracil is replaced by FU, is the same as that of normal MS2 RNA. The specific infectivity of this highly replaced FU phage is about 20% of normal.

1. Introduction

Halogenated pyrimidines have been shown to replace analogous bases in nucleic acids, leading to molecules with altered properties. BU, for example, replaces thymine in DNA (Dunn & Smith, 1954; Zamenhoff & Griboff, 1954) and the resulting BU-DNA has a greater buoyant density than normal (Meselson, Stahl & Vinograd, 1957). This property has been used extensively to study the fate and function of DNA molecules. BU is also mutagenic, presumably by inducing base-pairing errors during or after incorporation into DNA (Litman & Pardee, 1956; Freese, 1959). Whereas BU is a thymine analog, the related FU is incorporated into RNA in place of uracil (Horowitz & Chargaff, 1959). In the case of an RNA virus, replacement of uracil by FU leads to virus particles with increased buoyant density (Shimura & Nathans, 1964). Bacterial cells grown in the presence of FU produce modified proteins (Naono & Gros, 1960); this effect has been ascribed to base-pairing errors when aminoacyl transfer RNA binds to FU-containing messenger RNA on the ribosome (Champe & Benzer, 1962). The availability of an RNA bacteriophage containing FU affords an opportunity to explore further the effect of FU on the function of messenger RNA.

In this report we detail the method of preparation of coliphage MS2 (Strauss & Sinsheimer, 1963) containing FU (FU phage), previously outlined in a preliminary note (Shimura & Nathans, 1964), and present observations on the physical properties and infectivity of the analog-containing virus. In a later paper we shall describe

† Abbreviations used: FU, 5-fluorouracil; BU, 5-bromouracil; TCA, trichloroacetic acid; FUMP, 5-fluorouridine monophosphate; p.f.u., plaque forming units; T_m, melting temperature.

the process of infection with FU phage and the characteristics of cell-free protein synthesis in the presence of FU-containing RNA.

2. Materials and Methods

Growth of MS2: MS2 and its host *Escherichia coli* C3000 were obtained from R. L. Sinsheimer. *Escherichia coli* was grown in a salts–glutamate medium, containing /l., 1·33 g KH_2PO_4, 13·3 g K_2HPO_4, 2·0 g NH_4Cl, 3·0 mg $FeSO_4,7H_2O$, 0·40 g $MgSO_4,7H_2O$, 6·7 g monosodium glutamate, 10 g glucose and 10 mg thiamine hydrochloride. For growth of MS2, $CaCl_2$ was added to give a final concentration of 0·0005 M. In experiments with ^{32}P, 13 g/l. of tris was substituted for phosphate, which was present at a concentration of 6×10^{-4} M, and the pH adjusted to 7·2 with HCl.

In the preparation of MS2, cells at a concentration of about 8×10^8/ml. were generally infected at an input multiplicity of 10 and phage harvested at 60 min by a procedure based on that of Cooper & Zinder (1963) for coliphage f2. Infected bacteria were shaken with chloroform and incubated with 0·1 mg/ml. of egg white lysozyme. After 1 hr at room temperature, the lysate could be stored overnight at 4°C. 320 g of solid ammonium sulfate was added /l., and after being stirred for 30 min at 4°C, the suspension was centrifuged at 10,000 *g* for 20 min. The precipitate was suspended in 0·01 M-tris–HCl (pH 7·4)–0·01 M-magnesium acetate (tris–Mg) at room temperature (20 ml./l. of lysate) and pancreatic DNase added to give a concentration of 5 μg/ml. After standing at room temperature for 1 hr, the extract could be stored overnight at 4°C. The extract was centrifuged at 15,000 *g* for 10 min and the precipitate extracted with 10 ml. of tris–Mg four more times. The combined supernatant solutions were centrifuged at 100,000 *g* for 90 min and the pellets resuspended in tris–Mg. To each 3·3 ml. of phage suspension 2·1 g of CsCl were added and the solution centrifuged at 37,000 rev./min in the SW39 Spinco rotor for 20 hr. One-drop fractions were collected and where necessary the pooled phage fractions were dialyzed to remove CsCl. The density of CsCl fractions was determined by direct weighing of 0·025-ml. portions.

Assay of MS2 was carried out as described by Cooper & Zinder (1962) for f2. In all cases where lysates were assayed, the cells were first lysed with chloroform and lysozyme.

MS2 RNA was extracted from purified phage in 0·1 M-sodium phosphate (pH 6·8) by two successive treatments with 90% phenol at 4°C. The final aqueous layer was extracted 6 times with ether and bubbled with nitrogen to remove residual ether.

Percentage replacement of uracil by FU was determined on alkaline hydrolysates of ^{32}P-labeled FU-RNA by counting $UM^{32}P$ and $FUM^{32}P$ separated by electrophoresis on Whatman 3MM paper at 35 v/cm for 2 hr in borate buffer at pH 9·3 (Gordon & Staehelin, 1959).

Separation of 2′,3′-AMP and 2′,3′-GMP from alkaline hydrolysates of RNA was accomplished by ascending chromatography on acid-washed Whatman 40 paper in *n*-propanol–ammonium sulfate–0·1 M-sodium phosphate, pH 6·8 (600 g ammonium sulfate/l. of buffer, to which is added 20 ml. *n*-propanol).

Radioactivity was measured in a liquid-scintillation counter with 2,5-diphenyloxazole and 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene in toluene. [^3H]RNA samples were prepared by cold TCA precipitation after addition of carrier yeast RNA, and solution of the washed precipitate in hyamine. [^{32}P]RNA samples were washed and counted on Whatman 3MM filter paper discs.

Source of chemicals. 5-Fluorouracil was kindly supplied by Dr R. Duschinsky of Hoffman-La Roche, Inc., Nutley, N.J. CsCl was obtained from Penn Rare Metals, Inc., Revere, Pa., and Cs_2SO_4 from Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, N.Y. Tritiated FU was purchased from Nuclear Chicago, De Plaines, Ill. and tritiated adenosine from the New England Nuclear Corp., Boston, Mass. DNase and lysozyme were obtained from Worthington Biochemical Corp., Freehold, N.J.

3. Results

Effect of 5-fluorouracil on yield of infective virus

In a cycle of growth of MS2, FU inhibited the formation of infective phage (Shimura & Nathans, 1964). (Similar observations have been made with f2 by Cooper & Zinder (1962) and with f_{CAN1} by Davern (1964).) The extent of inhibition was strikingly dependent on the time of addition of FU; when the analog at a concentration of 30 μg/ml. was added soon after infection, there was a marked decrease in yield of infective phage, whereas addition of FU at later times during infection led to progressively greater yields of infective particles (Figs 1 and 2). In a separate experiment, the average burst size after addition of 30 μg FU/ml. at five minutes was 2·6, in contrast to a burst size of 60 when FU was added at 17 minutes, and 1500 in the absence of FU. As shown in Fig. 2, there is a rather precise time during the growth cycle after which addition of FU allows the formation of infective progeny. This time (13 minutes under the conditions of the experiment presented) corresponds to the time of onset of phage RNA synthesis determined with closely related RNA coliphages (Cooper & Zinder, 1963; Paranchych, 1963). We shall refer to the period of the growth cycle prior to the critical time, in which high concentrations of FU

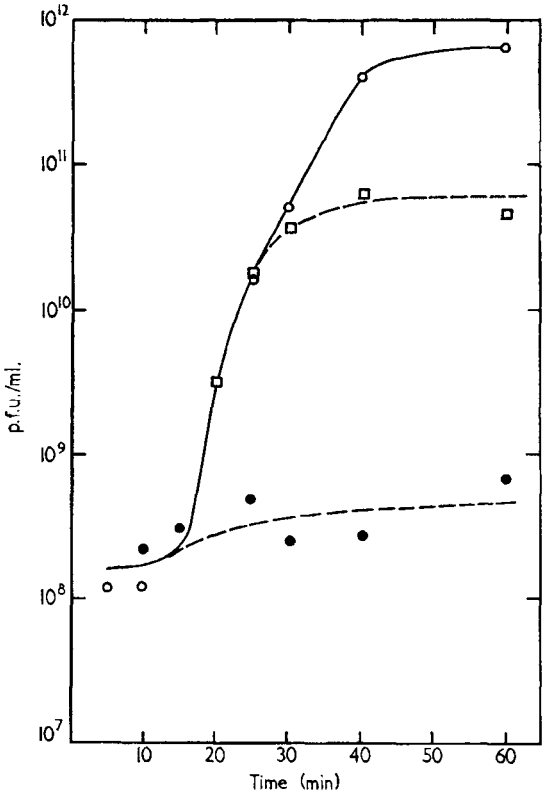

FIG. 1. Effect of FU on growth of MS2. *E. coli* C3000 was grown in salts–glutamate medium in a bubbler tube at 37°C. When the cells reached a concentration of 8×10^8/ml., they were infected at an input multiplicity of 9 and phage assayed at various times in chloroform–lysozyme treated portions (○). At 5 min (●) and 20 min (□) after infection, portions of the culture were added to FU to give a concentration of 30 μg/ml.

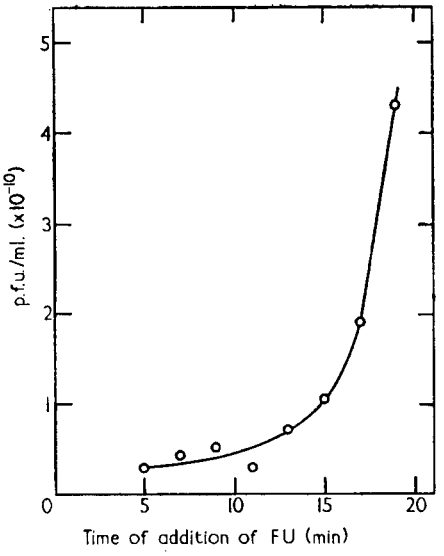

FIG. 2. Effect of time of addition of FU on yield of infective phage. Conditions of infection were the same as in Fig. 1. At the times noted in the Figure (minutes after infection), portions of the infected culture were added to FU to give a concentration of 30 μg/ml. After a total of 60 min growth in a bubbler tube, the cells were lysed and phage assayed.

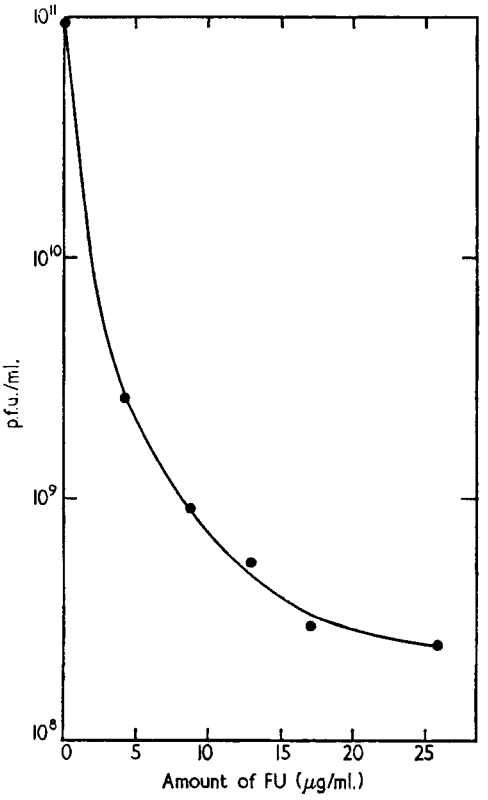

FIG. 3. Effect of early addition of FU at various concentrations on yield of infective phage. Conditions of infection were the same as in Fig. 1. 5 min after infection, portions of the culture were distributed into tubes containing FU to give the concentrations indicated. At 60 min, total phage was assayed in each sample.

essentially prevent the formation of infective particles, as the "early" period, and the period after this critical time as the "late" period.

In Figs 3 and 4 is shown the relationship between the concentration of FU in the medium and the final yield of infective virus for both the early and late additions of FU. For maximum early effect, about 20 μg/ml. of FU was required; and for maximum late effect, about 6 μg/ml. As shown in Fig. 4, there was little or no change in yield

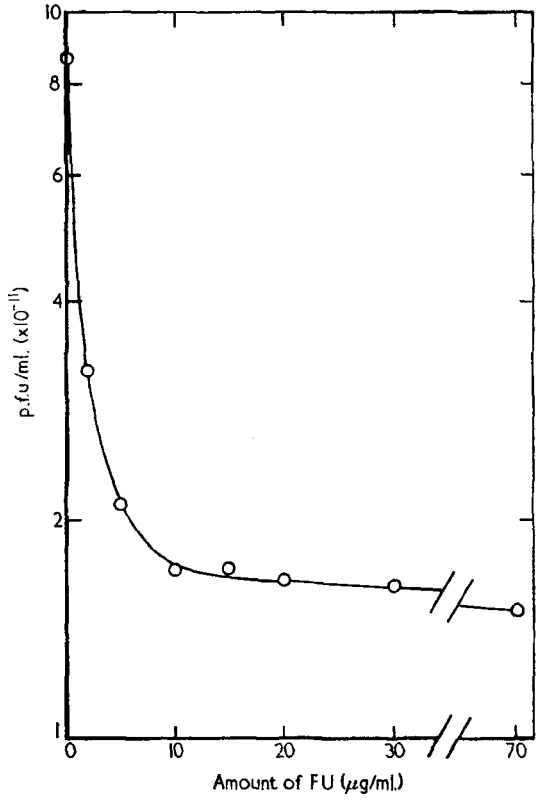

FIG. 4. Effect of late addition of FU at various concentrations on the yield of infective phage. Conditions as in Fig. 3, except that portions of the infected culture were transferred to tubes with FU at 17 min after infection.

of infective particles between FU concentrations of 6 and 70 μg/ml. These levels of FU correspond to approximately 77 and 80% replacement of uracil by FU, respectively (see below).

Effect of 5-fluorouracil on yield of physical virus particles

The results presented above refer only to the effect of FU on yield of infective particles. When the yield of physical virus particles was determined after early or late addition of 30 or 60 μg/ml. FU, analogous results were obtained: early addition of FU prevented formation of detectable progeny phage, whereas late addition of FU allowed phage to be formed (Fig. 5). As seen in Fig. 5(b), no virus was detectable either by absorbance at 260 mμ or by incorporation of radioactive FU when the latter was added early. After late addition of FU, however, two virus peaks differing in

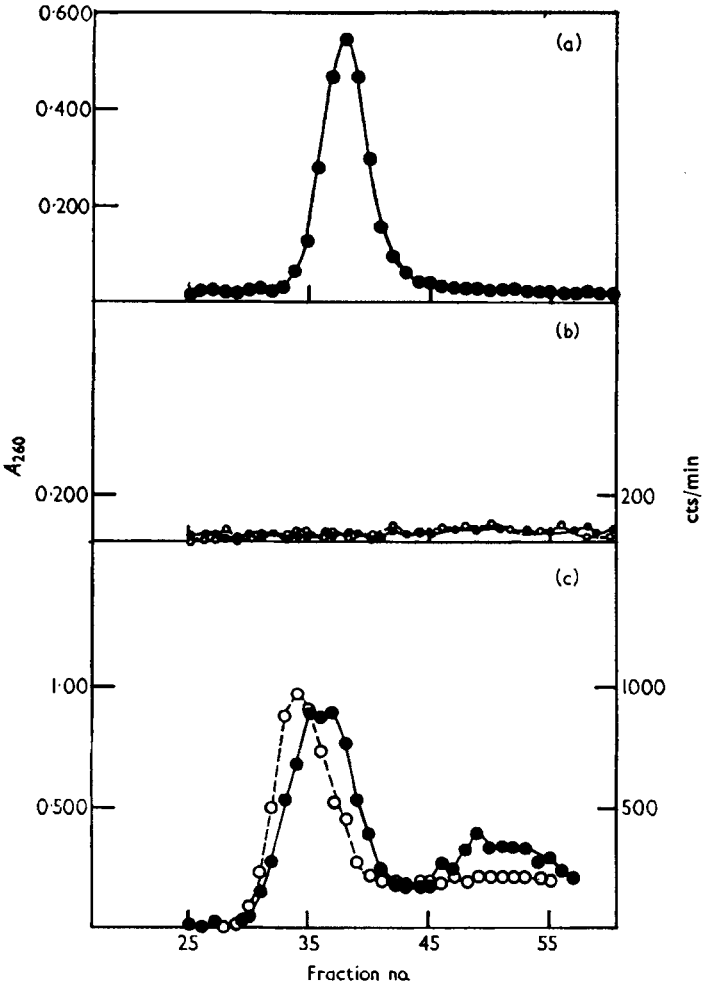

FIG. 5. Physical phage yield on addition of FU. Conditions of infection as in previous Figures. At 5 and 20 min, 10-ml. portions of the infected culture were added to bubbler tubes containing [^{3}H]FU (0·4 mc, 600 μg). After cell lysis at 60 min, phage titers were as follows: in the absence of FU, $2{\cdot}0\times10^{12}$ p.f.u./ml.; FU at 5 min, 1×10^{10}; FU at 20 min, $6{\cdot}1\times10^{11}$. The lysates were treated as described under Materials and Methods for purification of MS2. In the Figures the analysis of CsCl gradient fractions is presented: —●—●—, absorbancy; --○--○--, radioactivity. (a) No FU, A_{260} determined at a dilution of 1:200. (b) FU at 5 min, A_{260} determined at a dilution of 1:5 and radioactivity measured in cold TCA precipitate of 1/10 portion of each fraction. (c) FU at 20 min, A_{260} and radioactivity determined as in (b). Total fractions: (a) 98; (b) 86; (c) 94.

buoyant density in CsCl were detectable (Fig. 5(c)), the heavier corresponding to phage containing FU. (In many similar experiments in which [^{3}H]FU was added late, FU phage was consistently more dense than normal MS2, but a single broad A_{260} peak was often observed in the fractions collected, even though two distinct bands could be seen in the CsCl tube.) That the two phage peaks are distinct and represent, respectively, normal MS2 the RNA of which was synthesized prior to addition of FU and denser FU phage formed after addition of FU is shown by an experiment in which [^{3}H]adenosine was present prior to addition of FU, and

[^{32}P]orthophosphate was present after addition of FU. As seen in Fig. 6, normal phage containing [^{3}H]adenosine banded at a density of 1·424, whereas FU phage containing ^{32}P banded at a density of 1·436.

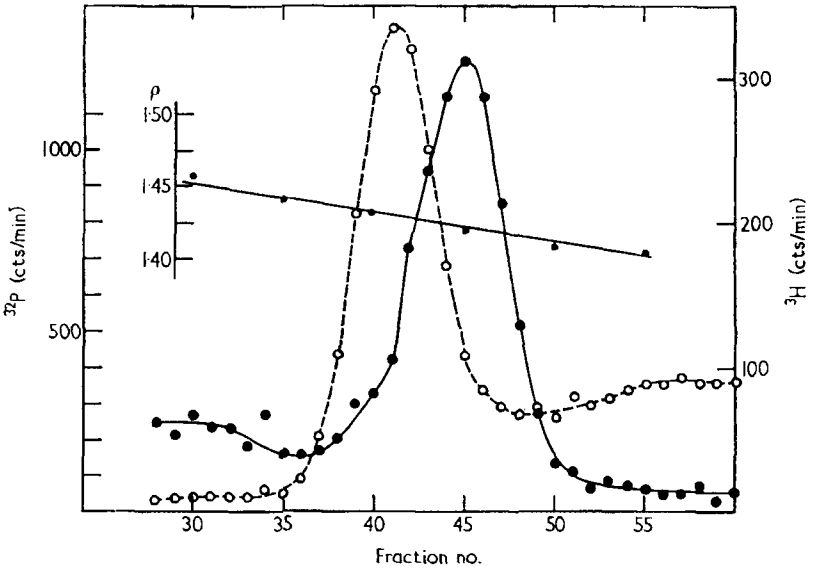

FIG. 6. Identification of normal and FU phage from the same growth flask. 100 ml. of *E. coli* C3000 growing in low-phosphate medium at 37°C was infected at an input multiplicity of 10. At 5 min, 60 μc [^{3}H]adenosine was added (200 μc/μmole) and at 16 min excess non-radioactive adenosine. At 18 min FU was added to give 60 μg/ml., and at 20 min 0·1 mc of [^{32}P]orthophosphate was introduced. Phage was harvested at 80 min ($1{\cdot}1 \times 10^{11}$ p.f.u./ml.) and purified as described in Materials and Methods. Shown in the Figure is the analysis of CsCl fractions for radioactivity (—●—●—, ^{3}H; --○--○--, ^{32}P) in RNA, measured on 1/80 of each fraction. A total of 107 fractions was collected. Buoyant density (ρ) is plotted as g/ml. (—●—).

The actual yield of total phage particles after late addition of FU was determined by measuring the incorporation of [^{3}H]adenosine into purified phage (Table 1). When FU was added 17 minutes after infection, at a concentration of either 6 or 30 μg/ml., the total yield of phage was about 50% of normal in each case. In the same experiment the yield of infective particles fell to 0·1 and 0·06 of the control, respectively (Table 1). Hence, the specific infectivity of the progeny phage is less than normal (see below).

TABLE 1

Yield of phage after late addition of 5-fluorouracil

Concn FU (μg/ml.)	Infective phage (p.f.u./ml.)	Physical phage (μg/mg reference phage)
0	$3{\cdot}4 \times 10^{12}$	120
6	$3{\cdot}2 \times 10^{11}$	59
30	$2{\cdot}1 \times 10^{11}$	70

Cells growing in 35 ml. salts–glutamate medium in a bubbler tube were infected with MS2 at an input multiplicity of 10. 1 min after infection 80 μc [^{3}H]adenosine was added (1 c/m-mole),

and at 17 min 5-ml. portions were transferred to tubes containing sufficient FU to give concentrations of 0, 6 or 30 μg/ml. The cells were lysed at 60 min and phage assayed. After addition of a 10-fold excess of non-radioactive lysate as a reference, phage was purified from each lysate as described in Materials and Methods, and the RNA radioactivity and A_{260} of the CsCl gradient fractions determined. Actual radioactivity was in the range of 40,000 to 80,000 cts/min. The specific activities of 2′,3′-AMP and 2′,3′-GMP from alkaline digests of the total RNA of each lysate were measured and found to be nearly identical in the three lysates ($2{\cdot}4 \times 10^6$ cts/min/μmole for AMP and $4{\cdot}1 \times 10^5$ cts/min/μmole for GMP). These values, together with the analytical data of Strauss & Sinsheimer (1963), were used to calculate the physical phage yield.

Appearance of a "light peak" in cesium chloride

In all experiments in which phage has been isolated following late addition of FU, a broad ultraviolet-absorbing "light peak" appeared in the CsCl gradient at a density of approximately 1·39 (see Figs 5(c) and 6). The amount of this material varied in different preparations, and often exceeded the quantity of normal and FU phage combined (Fig. 7). We have not observed a similar light peak in the absence of FU (Fig. 5(a)). The ultraviolet spectrum of this material, shown in Fig. 8, indicates that

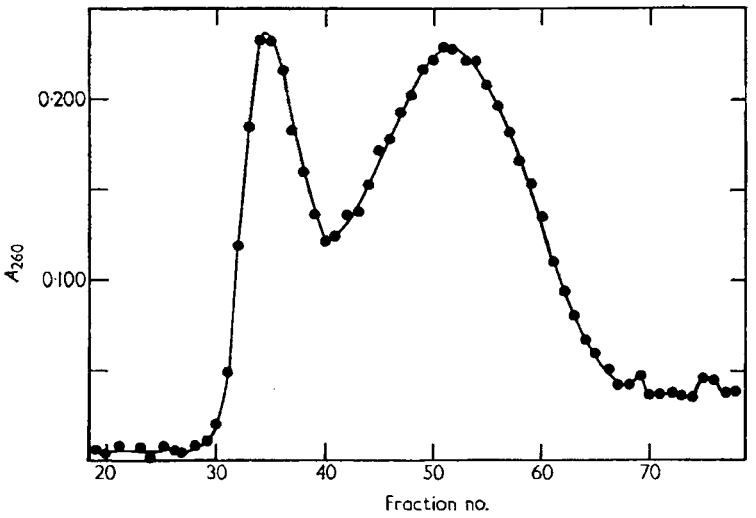

FIG. 7. Light peak appearing after late addition of FU. Conditions of infection as given in Fig. 1, except that growth proceeded in a flask containing 500 ml. of medium, and FU at 60 μg/ml. was added at 10 min after infection. (Since the entire growth cycle was found to be accelerated in a rapidly-shaken flask relative to the growth cycle in bubbler tubes, this time is equivalent to about 15 min in a bubbler tube.) In the Figure is shown the absorbancy at 260 mμ of 1:500 dilutions of the CsCl fractions. A total of 89 fractions was collected. Normal MS2 is expected to form a peak at fraction 39.

it has a higher protein:RNA ratio than normal MS2, as expected from its buoyant density. The observations that it contains FU-RNA (Figs 5(c) and 6) and that it reacts with antiserum to MS2 suggest that the light peak material is incomplete phage with fragments of RNA. More detailed studies are in progress to identify it with greater certainty.

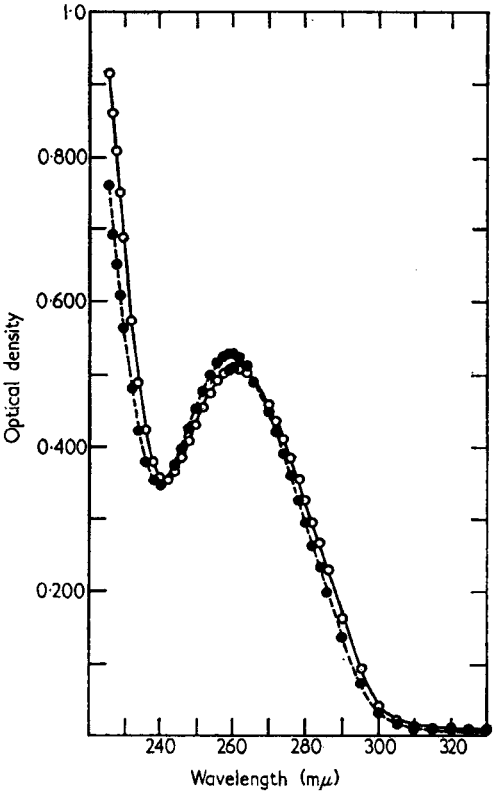

FIG. 8. Ultraviolet spectra of the "light peak" (—○—○—) and normal MS2 --●--●-- at pH 7.

Purification of FU phage by cesium chloride centrifugation

The greater buoyant density of FU phage compared to normal MS2 provides a method of purifying the analog-containing virus. By re-banding the denser fractions of the phage peak in CsCl, it has been possible to prepare FU phage the uracil of which is largely replaced by FU, free or almost free of normal MS2. Usually two runs in CsCl have been sufficient for this purpose (see Fig. 11(a)), provided the less dense fractions are excluded.

Density of FU-RNA

On the basis of the evidence presented so far, it is not possible to conclude that the high buoyant density of FU phage is due to altered density of its RNA. Direct examination in Cs_2SO_4 of the density of RNA extracted from purified FU phage, however, revealed that FU-RNA is considerably denser than normal MS2 RNA (Fig. 9). The density in Cs_2SO_4 of FU-RNA exceeded that of normal RNA by 0·031 g/ml., whereas the density in CsCl of the FU phage from which the RNA was extracted exceeded that of normal MS2 by 0·012 g/ml. This difference suggests that the high density of FU phage is due to the dense RNA which it contains.

Density of FU phage as a function of percentage replacement of uracil by 5-fluorouracil

FU phage containing ^{32}P was prepared by growing phage in the presence of FU

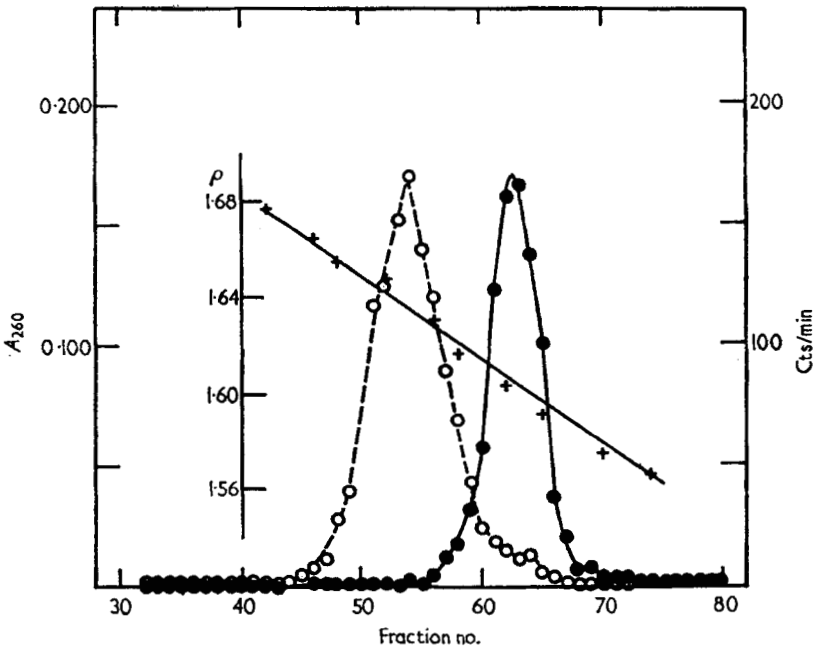

FIG. 9. Buoyant density of FU-RNA in Cs_2SO_4. [^{32}P]FU phage from the experiment shown in Figure 6 was re-centrifuged in CsCl, and after dialysis of the phage against 0·1 M-sodium phosphate buffer of pH 6·8, RNA was extracted. A sample of this RNA containing 2500 cts/min was mixed with 1 O.D. unit of normal MS2 RNA and the mixture centrifuged in Cs_2SO_4 (ρ = 1·60)–0·05 M-sodium phosphate (pH 6·8) at 37,000 rev./min in the Spinco SW39 rotor for 50 hr at a centrifuge setting of 40°F. 132 fractions (3·45 cc total) were collected. Absorbancy at 260 mμ (—●—●—) was measured at a dilution of 1:5, and radioactivity in RNA (--○--○--) was determined on 5/6 of each fraction; density, (—+—+—).

at concentrations of 1, 3, 6 and 60 μg/ml. FU was added at 14 minutes after infection and [^{32}P]orthophosphate at 16 minutes. Phage was purified as described in Materials and Methods; but prior to banding in CsCl, [^{3}H]tyrosine-labeled normal MS2 was added as a density reference. After determination of the density of each FU phage preparation, [^{32}P]RNA was prepared from each sample. The RNA was hydrolyzed in 0·3 M-KOH overnight at 35°C and the resulting nucleotides subjected to electrophoresis as described in Materials and Methods (amounts giving approximately 50,000 cts/min of each sample were applied to paper). As shown by radioautography, 2′,3′-UMP and a radioactive compound corresponding in mobility to that reported

TABLE 2

Relation of 5-fluorouracil content and density of phage

Concn FU (μg/ml.)	Percentage replacement of uracil by FU	Density difference †
1	12	0·002
3	47	0·005
6	77	0·009
60 ‡	80	0·010

† Density difference is the density of FU phage minus the density of reference MS2.
‡ Separate experiment.

for 2',3'-FUMP (Gordon & Staehelin, 1959) were clearly separated. The UMP and FUMP spots were cut out and counted to determine the percentage replacement of uracil by FU. The results, presented in Table 2, demonstrate the direct relationship between percentage replacement of uracil by FU and the buoyant density of the FU phage.

Sedimentation rates of FU phage and FU-RNA

Sedimentation rates of purified FU phage and FU-RNA were compared to those of normal preparations grown and purified at the same time. (We are indebted to Dr W. Moller for these determinations.) FU phage was grown in the presence of 60 μg/ml. of FU, at which concentration 80% of uracil is replaced by FU (see above). S_{20} values of the MS2 preparations measured in 0·1 M-sodium phosphate (pH 6·8) were 69·6 s for the normal and 70·4 s for FU phage. FU-RNA extracted from this phage preparation had an S_{20} value of 33·6 s in 0·01 M-tris HCl (pH 7·4)–0·01 M-magnesium acetate, whereas normal RNA had a value of 34·3 s in the same solvent. (This was the value of the major and fastest component; there was evidence of some breakdown of RNA in both preparations.) Sedimentation-velocity measurements thus indicate that there is no gross difference in the structure of FU phage or FU-RNA compared to normal.

Melting profile of FU-RNA

The melting profile of FU-RNA was examined, since a less ordered structure might contribute to the increase in buoyant density of FU phage and its RNA. As shown in Fig. 10, the melting profile of FU-RNA differed only slightly from normal; the T_m of each preparation was 61·5°C. In two separate preparations the maximum

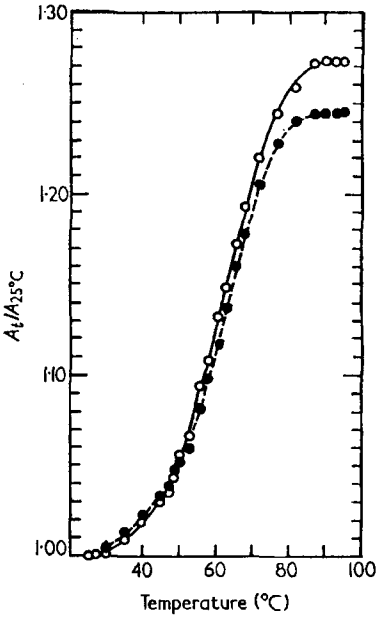

Fig. 10. Melting curves of normal and FU-RNA. Purified FU phage prepared in the presence of 60 μg FU/ml. and normal MS2 prepared in parallel were dialyzed in the same flask against 0·1 M-sodium phosphate buffer of pH 6·8. RNA was extracted from each phage preparation and the optical density at 259 mμ determined in a heated cell-holder at the temperatures indicated. Normal RNA, —○—○—; FU-RNA, --●--●--.

hyperchromicity of FU-RNA was 12 and 11% less than that of normal MS2 RNA prepared in parallel. The significance of this observation is not known.

Infectivity of FU phage

The infectivity of several preparations of purified FU phage was compared with normal MS2 prepared simultaneously either from the same growth flask or from a parallel flask. As indicated in Table 3, the specific infectivity of FU phage prepared in the presence of 15 to 60 μg/ml. (approximately 80% replacement of uracil by FU) is less than normal. Although there is variable loss of infectivity during purification,

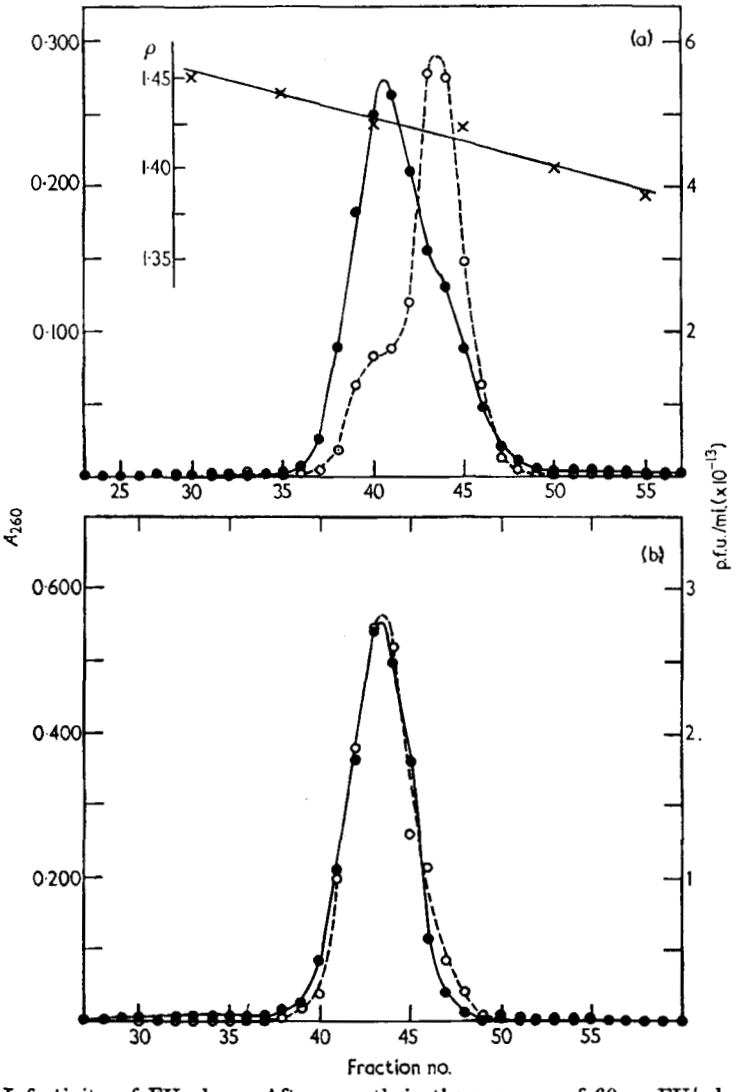

FIG. 11. Infectivity of FU phage. After growth in the presence of 60 μg FU/ml., phage was purified as described in Materials and Methods and the denser half of the CsCl gradient peak was re-centrifuged in CsCl (a). Fractions were titrated (--○--○--) and A_{260} determined at a dilution of 1:500 (—●—●—). Part of fraction 39 was again banded in CsCl (b). In (b) A_{260} was measured at a dilution of 1:5. Total fractions: (a) 83; (b) 93. Density, —×—×—.

TABLE 3

Specific infectivity of 5-fluorouracil phage

Experiment	Concn FU (μg/ml.)	Normal	p.f.u./o.d. unit FU	FU/normal
1	15	$1{\cdot}3\times10^{11}$	$3{\cdot}3\times10^{10}$	0·25
2	30	$2{\cdot}3\times10^{12}$	$6{\cdot}8\times10^{11}$	0·20
3	60	$6{\cdot}0\times10^{11}$	$1{\cdot}2\times10^{11}$	0·20
4	60	$2{\cdot}7\times10^{12}$	$1{\cdot}3\times10^{11}$	0·05

In experiments 1, 2 and 3, normal and FU phage were separated from the same lysate. In experiment 4, normal MS2 was prepared in a parallel flask.

the difference in specific infectivity between normal and FU phage prepared and purified together is a consistent finding. This difference in infectivity is seen most strikingly in an experiment in which fractions of the infective phage banded in a sharp peak at the expected density of normal MS2, whereas a smaller peak of infectivity corresponded to the A_{260} peak of FU phage (Fig. 11(a)). That this minor peak of infectivity was actually due to FU phage and not to smearing of the normal phage peak was shown by re-banding a portion of the FU phage; as seen in Fig. 11(b) the infectivity corresponded to the A_{260} peak of FU phage.

4. Discussion

Effect of 5-fluorouracil on phage development

The mechanism whereby high concentrations of FU added prior to a critical time in the growth cycle of MS2 inhibit the formation of physical phage particles is not known. It does not appear to be due to inhibition of thymidylate synthesis (Cooper & Zinder, 1962), to interference with synthesis of the new RNA-dependent RNA polymerase (Lodish, Cooper & Zinder, 1964), or to a side effect of FU on cellular metabolism, for example, cell wall synthesis (Tomasz & Borek, 1962), since only slightly later addition of FU leads to substantial yields of virus. The correspondence of this critical time to the onset of RNA synthesis in related phage systems (Cooper & Zinder, 1963; Paranchych, 1963) suggests that FU interferes with the formation or expression of new RNA molecules. For example, it may interfere with the formation or function of a replicative form (Weissmann, Borst, Burdon, Billeter & Ochoa, 1964; Kelly & Sinsheimer, 1964); or it may alter the expression of new RNA for the synthesis of coat protein or an essential enzyme at present undetected. In view of the continued synthesis of host RNA during phage development (Cooper & Zinder, 1962), one also cannot exclude an effect on host RNA.

The infectivity of FU viruses is of course relevant to the question of altered expression of FU-RNA (cf. Gordon & Staehelin, 1959; Munyon & Salzman, 1962; Davern, 1964). The fact that FU phage has only moderately reduced specific infectivity indicates that the infecting FU-RNA, even with 80% of its uracil replaced by FU, can serve as template for at least some active molecules of polymerase, and that at least some of the RNA made on this template functions in coat protein synthesis as well as in subsequent rounds of infection. However, we have found that the average burst size on infection with FU phage is reduced compared to normal both with

respect to infective progeny and yield of physical virus. Also, it has been reported recently that FU is mutagenic for poliovirus (Cooper, 1964) and for an RNA coliphage (f_{CAN1}) related to MS2 (Davern, 1964). It is therefore clear that the function of FU-RNA in the infected cell is abnormal.

Buoyant density of FU phage and FU-RNA

The increased buoyant density of FU phage appears to be due to an increased density of its RNA. There is a direct relationship between the degree of replacement of uracil by FU and the buoyant density of FU phage. It seems likely, therefore, that the substitution of fluorine for hydrogen is the primary cause of the altered density. This property of RNA which contains FU may prove useful in future investigations of RNA function.

We are grateful to Kenneth Bourne for able technical assistance. This research has been supported by grant number GM 10180 of the National Institutes of Health, United States Public Health Service. One of us (Y.S.) is a Fellow of the Damon Runyon Memorial Fund; another (R.E.M.) is a Post-Sophomore Scholar of the Johns Hopkins University School of Medicine.

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